De novo design and in vivo activity of conformationally restrained antimicrobial arylamide foldamers

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The emergence of drug-resistant bacteria has compromised the use of many conventional antibiotics, leading to heightened interest in a variety of antimicrobial peptides. Although these peptides have attractive potential as antibiotics, their size, stability, tissue distribution, and toxicity have hampered attempts to harness these capabilities. To address such issues, we have developed small (molecular mass <1,000 Da) arylamide foldamers that mimic antimicrobial peptides. Hydrogen-bonded restraints in the arylamide template rigidify the conformation via hydrogen bond formation and increase activity toward *Staphylococcus aureus* and *Escherichia coli*. The designed foldamers are highly active against *S. aureus* in an animal model. These results demonstrate the application of foldamer templates as therapeutics.

antibiotic | host defense peptide

Resistance to conventional antibiotics is rapidly increasing, posing a substantial threat to global public health (1). Recently, methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resistant *Staphylococcus epidermidis*, vancomycin-resistant enterococci (VRE), and ampicillin-resistant *Escherichia coli* have emerged as common nosocomial (hospital-acquired) infections (2–4). The escalating resistance to conventional agents has inspired a substantial research effort directed toward investigating the potential of antimicrobial peptides (AMPs). Although no mechanism of pharmaceutical intervention is devoid of problems associated with resistance, AMPs use a physical mechanism that targets the bacterial cell membranes, possibly decreasing the risk of resistance (5–7).

AMPs are found in a wide range of species, including: plants, frogs, worms, and humans (8, 9). These host-defense peptides are typically 20–50 residues in length and span several structural classes. Despite this diversity, they all tend to adopt highly amphiphilic topologies in which the hydrophilic and hydrophobic side chains segregate onto distinctly opposing regions or faces of the molecule. Electrostatic interactions between the positively charged AMPs and the negatively charged bacterial phospholipids provide an initial mode of interaction, whereas hydrophobic interactions allow the peptides to penetrate the cell membrane (10, 11), in some cases leading to depolarization of the bacterial membrane and cell death. AMPs with appropriate distributions of charged and hydrophobic residues are remarkably selective for killing bacterial cells relative to host cells (12, 13). This selectivity is a result of a fundamental difference between the lipid composition of bacterial membranes and that of eukaryotic membranes. Bacterial membranes are composed of ≈30% negatively charged phospholipids (phosphatidylglycerol), with phosphatidylcholine as their zwitterionic lipid (14), whereas the surface of a eukaryotic cell membrane is composed mainly of zwitterionic phospholipids (such as phosphatidylcholine, sphingomyelin phospholipids, and cholesterol).

Over the last decade, new classes of peptidic and nonpeptidic antimicrobial compounds with structures similar to those of cationic and facially amphiphilic host defense antimicrobial peptides have been extensively investigated as therapeutic agents. A number of studies reported antibiotics designed to follow the mechanism of natural AMPs, for example, peptides composed of α-amino acids (15–17), β-amino acids (18, 19), peptoids (20), aromatic oligomers (21–24), and synthetic polymers (25–27). Previously, we designed a series of arylamide foldamers that showed potential for both activity and selectivity (21). However, the compounds were not active and exhibited significant toxicity in animal models. Therefore, we altered the structural and physicochemical properties of the arylamide foldamers to improve their antimicrobial activity and selectivity against *S. aureus* and *E. coli*. Compared with magainin analogues (e.g., MSI-78) (28) these compounds have significantly enhanced selectivity and reduced toxicity.

Results

Design and Structure of an Arylamide Framework. In previous studies (21), we prepared compounds related to generic structure **1,** consisting of arylamides containing 2 1,3phenylene diamine units connected by a single isophthalic acid (Fig. 1*A*). A key feature in the design of these arylamides was a thioether moiety that provided a convenient point of attachment to the basic groups. The thioether also forms intramolecular hydrogen bonds (21, 22, 29–31) to neighboring amides, thereby restricting rotation about the N–C torsional angle between the amide nitrogen and the phenyl ring. However, the molecule retained significant torsional flexibility associated with ω, the torsional angle connecting the amide carbon and the phenyl group of the isophthalic acid ring.

Herein, we increase the rigidity of the arylamide scaffold using a 4,6-dialkoxy-substituted isophthalic acid linker to form intramolecular O···H–N hydrogen bonds (generic structure **2**), thereby restricting rotation around the aryl–CO bond. The ether substituents also provide convenient points from which to attach

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Conflict of interest statement: W.F.D. is a member of the scientific advisory board of PolyMedix, a company that is conducting clinical trials of molecules related to the compounds described in this manuscript.

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Data deposition: The atomic coordinates have been deposited in the Cambridge Structural Database, Cambridge Crystallographic Data Centre, Cambridge CB2 1EZ, United Kingdom (CCDC reference no. 721880).

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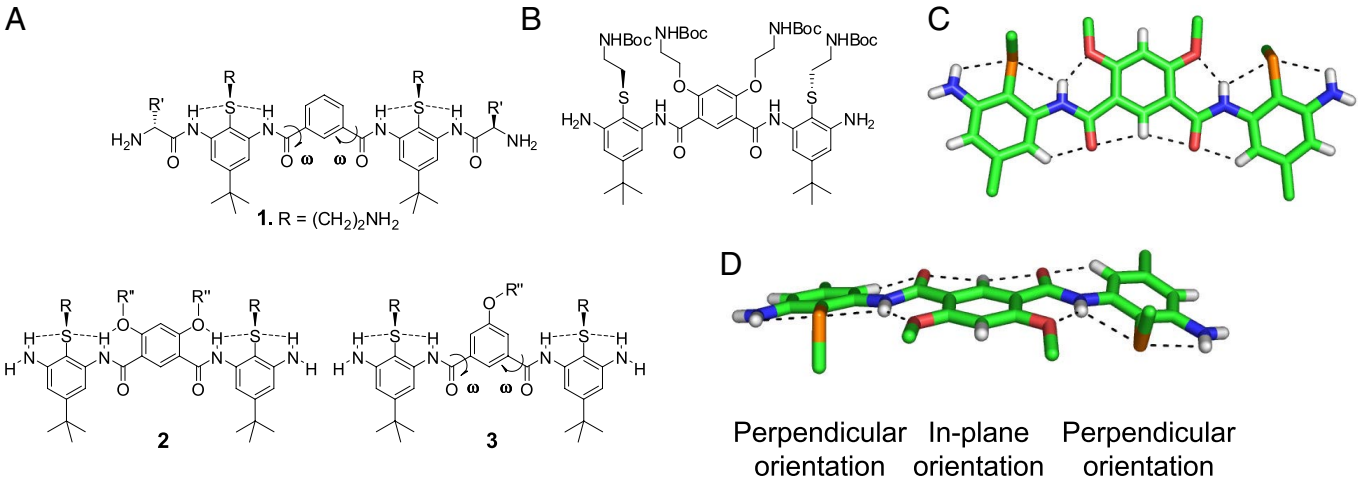


Fig. 1. Structures of arylamide foldamers. (*A*) Generic structures of conformationally restrained antimicrobial arylamide foldamers 1–3. (*B–D*) Chemical structure (*B*) and X-ray crystal structure (*C* and *D*) showing the network of hydrogen bonds through arylamide foldamers and the projection of the side chains from the plane of the ring [the side chains have been truncated; C (green), O (red), N (blue), polar H (white), and S (yellow)].

side chains (R″). To evaluate the conformational properties of this scaffold, we synthesized and determined the crystal structure of an arylamide foldamer with this substitution pattern (Fig. 1 *B–D*). In agreement with previous calculations (29–31), the tandem thioether and ether groups serve as conformational directing elements that restrict rotation around the aromatic ring–N bond and aromatic ring–C(═O) bond, respectively. The thioether groups form intramolecular hydrogen bonds to adjacent amide and terminal amine protons (N···S distances = 3.02, 2.99, 2.99, and 2.97 Å); the 2 ether groups of the isophthalic amide linker also hydrogen bond to adjacent amide protons (N···O distances = 2.66 and 2.65 Å), forming 3-center intramolecular hydrogen bonds that rigidify the entire arylamide trimer. On the opposite face of the foldamer, we observed close proximity, typical of CH hydrogen bonds, between aryl CH-protons and amide oxygens (H···O distances = 2.22, 2.37, 2.37, and 2.32 Å). Although the angles are not optimal for a C–H···O hydrogen-bonded interaction (32), this feature might be important for maintaining the overall planar conformation. Thus, the overall network of consecutive N–H···S, N–H···O, and C–H···O interactions stabilizes the desired conformation and provides a platform on which to design a molecular recognition system with adjustable specificity.

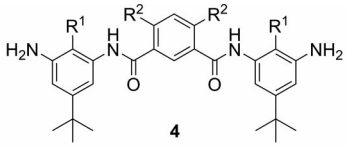
To provide some measure by which to gauge effectiveness of the conformational restraints introduced into **2**, we also prepared a series of compounds (generic structure **3**) in which a single alkoxy group was introduced at the 5-position of the isophthalic acid ring. Although a direct comparison is complicated by the presence of two R″ groups in **2,** while there is only one in **3**, distinct structure-activity relationships (SAR) were found for the two series of compounds (Fig. 1A).

Antimicrobial and Hemolytic Activity. The charge and hydrophobicity of the pendant functional groups were systematically varied (Tables 1–4) to determine how these parameters relate to activity in these 2 series of compounds. Furthermore, the terminal aniline amino groups in **2** and **3** (Fig. 1*A*) are very weakly basic and unlikely to be charged at neutral pH when bound to a membrane. Thus, we investigated the effect of converting these groups to the corresponding guanidines. The antimicrobial activities of compound **4a–g**, **5a–c**, and **6a–d** were determined as the minimal inhibitory concentration (MIC) required to fully inhibit the growth of *S. aureus* ATCC27660 (Gram-positive) and *E. coli* D31 (Gram-negative) (Tables 1–4), along with hemolytic activity (HC_{50}) against human erythrocytes. The MIC values are reproducible to within a factor of 2, whereas there is on the order of 10% to 20% error in the HC_{50} values depending on the source of human erythrocytes.

We observed an increase in both affinity and selectivity upon increasing the rigidity of the molecule (Table 1). As expected, the introduction of 2 methoxy groups in compound **4b** increased the antimicrobial activity 17- and 8-fold against *S. aureus* and *E. coli*, respectively, when compared with **4a**, which lacks these conformation-directing elements. Simultaneously, the hemolytic potency was decreased 10-fold (higher HC_{50}) for an overall 170 and 80-fold gain in selectivity (HC_{50}/MIC). These changes are attributed primarily to the more rigid structure of **4b** induced by introduction of the ortho methoxy groups to the amides, although other factors including changes in hydrophobicity may also be important.

To determine the effect of the overall charge, we prepared compound **4c**, in which 2 aminoethyl groups replace the methyl groups of **4b**. This substitution resulted in retention in activity

Table 1. The effect of conformational restriction and guanidinylation of 4,6-disubstituted arylamide foldamers



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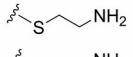
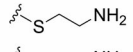
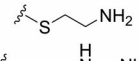
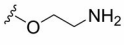
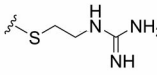
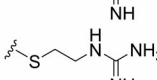
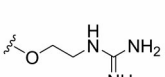
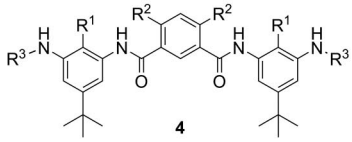
Compound	R^1	R^2	MIC (μM)* *S. aureus*†	MIC (μM)* *E.coli* D31	HC_{50} (μM)‡ erythrocyte
4a		H	15	7	14
4b			0.9	0.9	145
4c			0.7	5	593
4d			1.2	5	342
4e			2.3	0.6	328

*MIC, minimum inhibitory concentration.
†Tetracycline- and streptomycin-resistant.
‡Hemolytic concentration (50% lysis of human red blood cell).

Table 2. The effect of guanidine for amine replacements of 4,6-disubstituted arylamide foldamers



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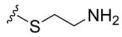
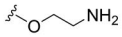
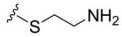
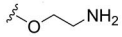
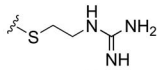
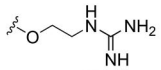
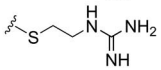
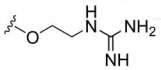
Compound	R^1	R^2	R^3	MIC (μM) *S. aureus*	MIC (μM) *E.coli* D31	HC_{50} (μM) erythrocyte
4c			H	0.7	5	593
4f				0.5	2.1	442
4e			H	2.3	0.6	328
4g				0.5	0.5	229

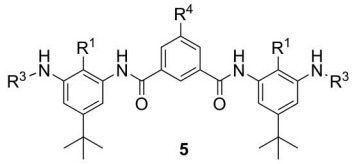
against *S. aureus*, although it decreased the potency ≈5-fold against *E. coli*. However, this modification also decreased the toxicity against red blood cells (HC_{50} = 0.6 mM), resulting in a very large (850-fold) selectivity for *S. aureus* versus human erythrocytes. Conversion of the alkyl-amino groups of **4c** to guanidines led to successively greater loss in activity against *S. aureus* in **4d** (with 2 guanidines) and **4e** (with 4 guanidines). On the other hand, these modifications increased the activity against *E. coli*. Simultaneously, these changes tended to slightly decrease the HC_{50} values, indicating that the compounds had become less selective for *S. aureus* and more selective for *E. coli*.

The weakly basic aniline amino groups were guanidinylated to introduce a positive charge at these positions (Table 2). This modification to **4c** did not significantly affect the antimicrobial or hemolytic activity (**4f**). On the other hand, guanidinylation of **4e** gave rise to a 4-fold increase in antimicrobial activity (**4g**), without significantly affecting the activity against *E. coli* and the hemolytic potency. Thus, in contrast to data shown in Table 1, we observed that guanidinylation of the terminal amine (R^3) resulted in a modest improvement in antimicrobial activity and selectivity against both bacteria.

An entirely different SAR arises from consideration of the less conformationally restricted arylamides with a 5-monosubstituent at the isophthalic amide (Table 3). Similar to the effect on 4,6-disubstituted foldamer **4b**, the introduction of a positively charged aminoethoxy group in **5a** decreased toxicity toward erythrocytes without significantly affecting antimicrobial activity. Interestingly, although the guanidinylation of the 4,6-disubstituted foldamers tended to decrease their antimicrobial activity against *S. aureus*, the 5-monosubstituted compound **5b** exhibited an unexpected 30-fold increase against *S. aureus*. This same substitution had no effect on the antimicrobial activity against *E. coli*, nor did it significantly change the hemolytic potency. Furthermore, in contrast to the results from **4e** and **4g**, guanidinylation of terminal aniline groups of **5b** (to give **5c**) led to a 3-fold decrease in the activity against *S. aureus*, a 40-fold increase against *E. coli*, and decreased toxicity toward erythrocytes. Thus, the effect of guanidinylation on the 2 series of compounds is strikingly different, presumably reflecting difference in overall charge as well as flexibility.

Finally, we explored additional substitutions (Table 4) into the conformationally constrained 2,4-dialkoxy isophthalic acid scaffold series of compounds. Based on previous work in which *N*-acyl groups had been shown to enhance activity of arylamides (21), we prepared compound **6a**, in which a guanidino-pentanoyl side chain was appended to the terminal amines. The activity of this compound was improved further by decreasing its overall charge through the replacement of the 2 aminoalkyl side chains with methyl ethers. We also explored the introduction of fluoroalkyl subsituents (33) by replacing the *t*-butyl groups with less hydrophobic trifluoromethyl groups in compound **6c**. Finally, in previous studies, a pyrimidine ring was used to control the conformation of antimicrobial arylamides in a manner similar to the dialkoxy

Table 3. The effect of guanidinylation of 5-monosubstituted arylamide foldamers



5

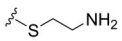
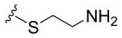
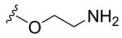
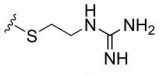
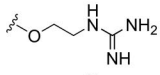
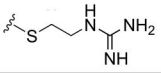
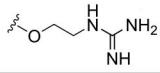
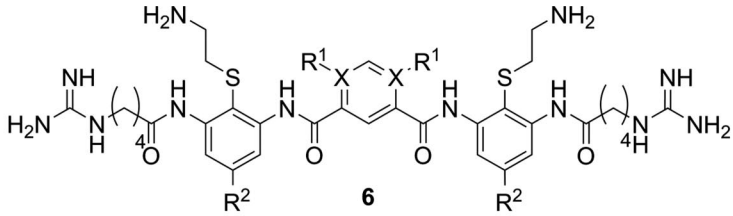
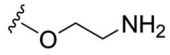
Compound	R^1	R^3	R^4	MIC (μM) *S. aureus*	MIC (μM) *E.coli* D31	HC_{50} (μM) erythrocyte
4a		H	H	15	7	14
5a		H		6	12	109
5b		H		0.2	11	97
5c				0.5	0.3	299

Table 4. Antimicrobial and cytotoxic activities of compounds 6a–d



6

Compound	X	R^1	R^2	MIC (μg/mL)				EC_{50} (μg/mL)*		HC_{50} (μg/mL)
				E.coli	*S.aureus*	*P.aeruginosa*	*K. pneumoniae*	3T3	HepG2	erythrocyte
6a	C		*t*-Bu	3.1	3.1	100	12	NA	NA	339
6b	C		*t*-Bu	1.6	1.6	25	6	>710	>710	NA
6c	C		CF_3	3.1	0.2	13	3.1	>2000	>2000	71-101
6d	N	–	CF_3	0.4	0.05	1.6	1.6	113	341	56

*MTS viability assay that measures dehydrogenase enzymatic activity in metabolically active cells and defines EC_{50} values representing compound concentrations that cause 50% lethality.

substituents in **4c** (24), prompting the synthesis of pyrimidine-containing compound **6d**. In vitro, compounds **6c** and **6d** were highly activity against a variety of Gram-positive and Gram-negative bacteria (Table 4). They also exhibited minimal toxicity to mammalian cells as measured using erythrocytes (HC_{50}), mouse 3T3, and human HepG2 cells. Comparison of the EC_{50} and HC_{50} values with the MIC for *S. aureus* indicated the high selectivity for bacteria over mammalian cells (250 to >20,000).

Cytoplasmic Membrane Permeabilization and Bacterial Killing. We examined the abilities of the arylamide foldamers, **4d**, **4e**, and **5b**, to depolarize *S. aureus* membranes, using the membrane potential-sensitive dye $DiSC_3$ (5). The distribution of this dye between the cell interior and the medium depends on the membrane potential gradient (34, 35), leading to an increase in fluorescence intensity in response to a loss of membrane potential. The potassium-selective ionophore valinomycin was used as a positive control. Maximum fluorescence was observed within 10 min after the addition of the arylamide compounds in a dose-dependent manner (Fig. 2*A*). We did not expect to see a perfect correlation between in vitro efficacy and ability to depolarize membranes, given the 2-fold error associated with determination of the MIC values and the limited spread of MIC values for this set of compounds. However, it is reassuring to note that the compound with the lowest MIC was most active in this assay, and the least active compound showed the least tendency to depolarize membranes.

For all compounds, the concentration required to cause membrane depolarization (after 10 min) was significantly higher than the MIC values, possibly because of differences in the assay conditions. The depolarization assay was conducted in 100 mM KCl with a bacterial density (OD_{600} = 0.02) 20-fold greater than the MIC assay. Thus, we evaluated the extent of cell death under the conditions of the fluorescence assay. After the fluorescence measurements, the cell suspension was diluted (30 min after addition of compound) and plated and the colony-forming units (CFUs) determined (Fig. 2*B*). The number of viable bacteria decreased in a dose-dependent manner over the same concentration range (≈1–20 μg/ml) that caused rapid depolarization of the membrane. Interestingly, although significant membrane depolarization occurred in this concentration range, full depolarization after 10 min did not appear to be necessary to elicit cell death after plating of the bacteria.

Resistance Study. To investigate the potential for bacteria to develop resistance against the antimicrobial activity of the foldamers, *S. aureus* was serially passed in sublethal concentrations of **4e**, and new

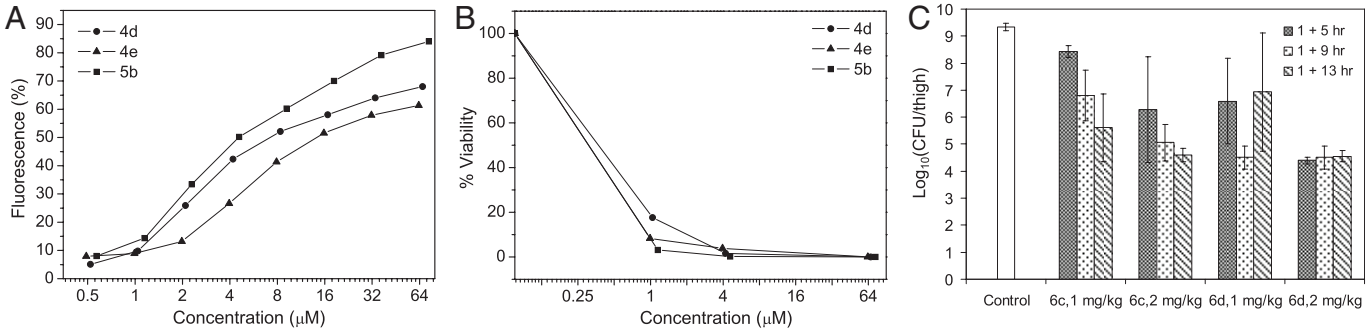


Fig. 2. In vitro and in vivo activities of arylamide foldamers. (*A*) Depolarization of the cytoplasmic membrane of *S. aureus*, assessed by the fluorescence intensity of membrane potential-sensitive dye $DiSC_3$ (34, 35). (*B*) Survival of *S. aureus* in the presence of compounds **4d**, **4e**, and **5b**. (*C*) In vivo efficacy of **6c** and **6d** in thigh burden infection model. Neutropenic mice (n = 4 per group) were inoculated in the posterior thigh muscles with *S. aureus* ATCC13709 at 1×10^6 CFU per thigh and then treated with **6c** and **6d** (1 or 2 mg/kg per dose) by i.v. bolus injection in the tail vein at 1 and 5, 1 and 9, or 1 and 13 h after infection.

MIC values were measured at 24-h intervals. Compound **4e** was chosen for this study because it had intermediate activity and was available in sufficient quantities. As a positive control, parallel cultures were exposed to serial 2-fold dilutions of ciprofloxacin and norfloxacin, which are 2 broad-spectrum fluoroquinolones (36). No significant change in the MIC occurred for **4e** over the entire 16 passages, whereas an increase in the MIC is readily observed by passage 6 for both ciprofloxacin and norfloxacin (supporting information (SI) *Appendix*). These results demonstrate that it is not particularly easy for *S. aureus* to evolve resistance to this arylamide. We are currently conducting more extensive studies at lower and higher drug concentrations to assess more fully the ability of various bacteria to evolve resistance the arylamides in vitro.

In Vivo Mouse Thigh Burden Infection Model. The development of membrane-active antimicrobial peptides as systemic agents for treatment of bacterial infections has been beset with difficulties associated with toxicity and tissue distribution, and few molecules have been shown to be active in vivo (37–40). A widely used animal model for evaluating antimicrobial activity of preclinical compounds (41) is the thigh burden model, in which the thigh muscle of neutropenic mice is inoculated with bacteria, followed by i.v. administration of the compound. Although complete pharmacokinetic and efficacy studies are beyond the scope of this manuscript, we report our initial findings here.

Compounds **4c** and **4e** provided partial protection against infection with *S. aureus* (*SI Appendix*), although the degree of protection at the highest tolerated dose was less than that conferred by successful antibiotics. Much greater activity was observed for compound **6c** and **6d**, which were tolerated at doses as high as 20 mg/kg (single i.v. bolus injection). Compound **6c** showed significant in vivo activity in the thigh burden model at doses of 10 mg/kg, 2 mg/kg, or 1 mg/kg when administered twice with a 6-hour interval between injections. At the highest dose, a 4-$\log_{10}$ decrease in CFU was observed (*SI Appendix*). For comparison, vancomycin typically produces a similar 10^4 to 10^5 reduction in viable CFU of *S. aureus* ATCC 13709 at its maximally efficacious i.v. dose of 30 mg/kg in this model. The efficacy of compound **6b** could be enhanced by increasing the time between doses to 13 h; 2 doses of 1 mg/kg or 2 mg/kg gave rise to a 10^4- to 10^5-fold reduction in CFU (Fig. 2*C*). Compound **6d** also showed excellent activity in this assay, although the degree of inhibition did not show the same time dependence observed for **6c**. At 2 mg/kg, compound **6d** showed excellent activity (10^5 reduction in viable CFU of *S. aureus* ATCC 13709) irrespective of the time between doses. At 1 mg/kg, the compound was only partially active, and considerable variability was observed.

Discussion

We have used arylamide foldamers to mimic the activities of antimicrobial peptides. These compounds are not only of potential use as systemic antibiotics but might also help elucidate the features required for antimicrobial activity. Like antimicrobial peptides, the arylamides cause rapid depolarization of the membrane in a concentration-dependent manner at concentrations close to those required to the MIC. Interestingly, only partial depolarization is required to lead to cell death, which occurs in a slower process that can take up to several hours at concentrations near the MIC (*SI Appendix*). Similar behavior has been observed for some AMPs, which show little membrane depolarization at concentrations that stopped growth (34, 35, 42). This behavior suggests that many AMPs and AMP mimics act by mixed mechanisms; at high concentrations they disrupt membranes sufficiently to lead to cell death, whereas at lower concentrations, other slower mechanisms become important. We are currently investigating the mechanisms of the antimicrobial arylamides, particularly the extent to which the rapid partial disruption of bacterial membranes is related to the slower processes that ultimately cause cell death.

The structure/activity relationships in this series of compounds illustrate the balance of forces required for activity. The arylamides are too short to span the hydrophobic length of the bilayer, and coarse-grained molecular dynamics simulations (43) suggest they work in part by a mechanism resembling the "carpet" mechanism of AMP activity (12). To reach their membrane targets, they must penetrate various physical barriers in Gram-positive and Gram-negative bacteria. Their relatively small size and conformationally constrained structures might aid in this process. Once they gain access to the cytoplasmic membrane, they must then bind with a sufficiently favorable free energy of association to allow disruption of the bilayer. Hydrophobic and electrostatic interactions appear to drive these interactions, but the overall hydrophobicity and charge must be carefully optimized to provide a sufficient driving force for binding while minimizing toxicity, aggregation, and nonspecific binding. Increasing the hydrophobicity beyond a certain threshold can lead to compounds with poor water solubility and toxicity toward mammalian cells. For example, we previously found that inclusion of a hydrophobic t-butyl substituent on the arylamide ring gave rise to good activity in vitro. Here, we find that substitution of this large hydrophobic substituent with a smaller trifluoromethyl group was tolerated without loss of antibacterial potency, while minimizing toxicity in vivo.

Many studies have explored the relationship between conformational flexibility and activity. Flexible compounds can be highly potent, possibly because membranes induce an amphiphilic conformation in the bound state (25, 40, 44). In such cases, additional electrostatic and hydrophobic interactions might be necessary to compensate for the loss in conformational entropy of binding in such compounds. Thus, the conformationally rigid compound **4b** was highly active, although it had only 2 strongly basic side chains, whereas the less rigid compounds **5b** and **5c** required 3 and 5 positively charged groups, respectively, to provide comparable activity. It is also interesting to note that quite different SARs emerged from the 2 different series of compounds, suggesting that they might have subtly different conformations or modes of binding. Also, although good activity in vitro could be obtained for each of the scaffolds investigated here, only the scaffolds the largest number of potential hydrogen-bonding conformational restraints gave good activity in vivo. Possibly, the toxicities seen in the more flexible molecules are associated with conformations that are distinct from those required for antimicrobial activity.

It is particularly encouraging that the compounds show good in vivo activity at doses significantly lower than the maximal tolerated dose. Unexpectedly, compound **6c** showed enhanced activity with an increasing delay time between doses. This effect might be related to its tissue distribution and pharmacokinetics, which have not yet been investigated. Alternatively it might have a mechanism similar to some AMPs, which can act indirectly through modulation of the host immune system (45). Thus, the increasing efficacy of **6c** with longer delays between doses might be associated with the priming of an immune response, which is then augmented by the second dose. This explanation appears less likely, because the animals are neutropenic, and the time frame covers only 24 h; conditions that are suboptimal for immune priming to a level that could account for the magnitude of efficacy. Furthermore, **6d** failed to show any statistically significant differences in activity with different dosing intervals when administered at either 1 mg/kg or 2 mg/kg. Pharmacokinetic analysis also showed that after i.v. injection of **6d** in mice, the serum levels of drug reached concentrations greater than the MIC (*SI Appendix*).

Finally, this work illustrates the potential of foldamers for the development of pharmaceuticals. Beginning with AMPs of molecular masses in the range of 2,000–5,000 Da, it has been

possible to design highly active arylamides with molecular masses in the range of 600–1,000 Da. These compounds might help address an urgent need for mechanistically novel drugs to combat the increasing threat posed by antibiotic-resistant bacteria. The in vivo activities seen for **6c** and **6d** are comparable with that of vancomycin at its maximum tolerated dose. Indeed, the good efficacy/safety ratios and broad-spectrum antibiotic activity seen in this study suggest mimics of AMPs might be excellent candidates for i.v. antibiotics

Methods

Method for determination of antimicrobial activity, toxicity, lipid depolarization, resistance, and in vivo antimicrobial activity can be found in *SI Appendix*. Synthetic details and crystallographic structure determination can be also found in *SI Appendix*.

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